groscopic), bromide (hygroscopic) or iodide (non-hygroscopic but unstable) salts. The allylically substituted 7-methoxy-1,3,5-cycloheptatriene,[5a] by giving with trityl bromide in sulfur dioxide only tropenium bromide and trityl methyl ether, shows methoxide instead of hydride exchange.

With more complete spectral data now available, it is evident that both predicted bands (4.33 ev, weak; 6.37 ev, intense)[6] for tropenium ion agree reasonably well with observed values (4.54 ev, 4350; 5.73 ev, 41,000). Results to date indicate that substituent effects on both $^1E_{1u}$ and $^1E_{3u}$ bands of substituted tropenium ions are in accord with those on $^1E_{1u}$[7] and $^1B_{1u}$, but not $^1B_{2u}$, bands of monosubstituted benzenes and are interpretable in terms of highly polar excited states for the ions.

Additional hydride exchange studies on tropenium and other systems for preparative and theoretical purposes are in progress.

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AN INTERMEDIATE IN THE DEACETYLATION OF MONO-ACETYL-δ-CHYMOTRYPSIN HAVING THE PROPERTIES OF ACETYL-IMIDAZOLYL[1]

Sir:

In a previous communication,[2] evidence was presented which indicated that upon acetylation of δ-chymotrypsin by *p*-nitrophenylacetate, there was no detectable change in the spectral characteristics of the enzyme in the region of 245 mμ. This region was studied carefully since a postulated intermediate in the reaction, acetyl-imidazolyl-,[3,4]

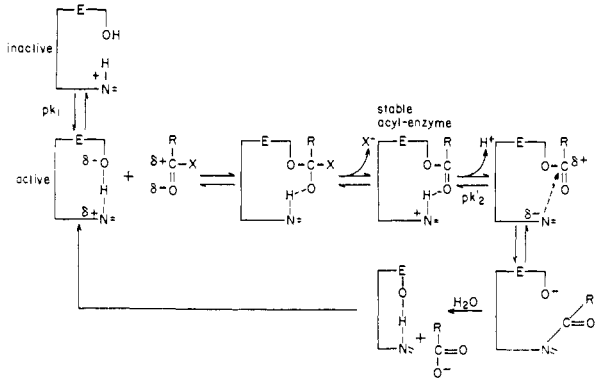

Fig. 1.—Proposed mechanism of enzymatic hydrolysis.

possesses a characteristic absorption maximum at this wave length.[4] Recently, a mechanism of reaction of δ-chymotrypsin with NPA has been proposed[5] which accounts for this observation and several others: (1) The stability of monoacetyl-δ-chymotrypsin at low *p*H[6] (2) a difference of 0.7–0.8 *pK* unit for acetylation and deacetylation of the enzyme[6] (3) the reversible sensitivity of the acetylation and deacetylation reactions to denaturation by urea,[2] (4) the reversible loss of reactivity of the acetyl with hydroxylamine in urea.[2] This scheme is presented in Fig. 1 with certain modifications, the evidence for which will be presented below.

A solution of monoacetyl-δ-chymotropysin (1.95 mgm./ml.) at *p*H 3.5 was allowed to deacetylate at *p*H 9.0 in a Beckman DK-1 spectrophotometer which was set at 245 mμ. The resulting record of ΔE_{245} with time is seen in Fig. 2, curve 1; a control

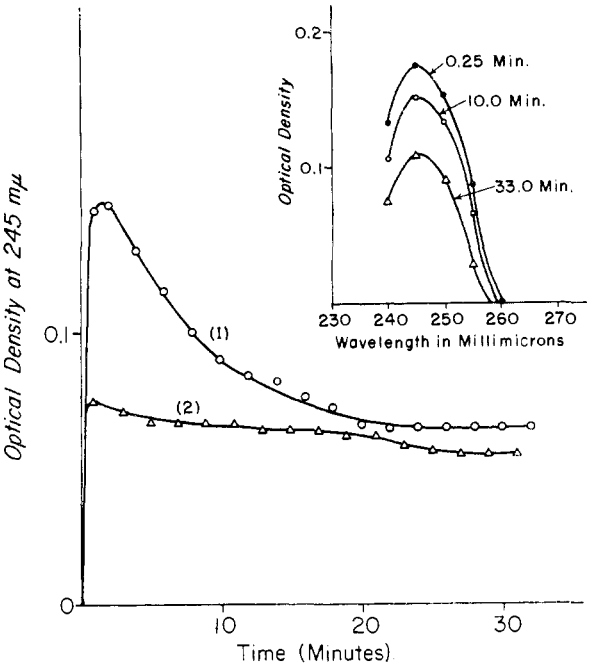

Fig. 2.—Both sample and reference cuvettes in a Beckman DK-1 spectrophotometer contained 3.0 ml. of the same solution of acetyl-δ-chymotrypsin[7] at *p*H 3.5; the temperature was 10.0 ± 0.2°. The *p*H of the sample was raised to 8.9 ± 0.1 by adding a small aliquot of a triethylammonium-ammonium acetate buffer on a plunger type rapid mixer.

of non-acetylated δ-chymotrypsin is seen in curve 2. Curve 2 was also followed when a portion of the acetyl-δ-chymotrypsin was allowed to deacetylate at *p*H 7.2 for 15 minutes at room temperature and then adjusted to *p*H 3.5 with acid before the reaction.

It is clear that only in the case of acetyl-δ-chymotrypsin is there the rapid formation of a species absorbing at 245 mμ which decreases slowly in concentration with time. The control curve indicates that part of the initial increase at 245 mμ is due to a non-specific, *p*H dependent, spectral change in the enzyme.

(1) The authors wish to acknowledge the financial support of grant No. RG-4617 from the National Institutes of Health, U. S. Public Health Service.

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When deacetylation was allowed to occur at the same *p*H but at 5.0 ± 0.2° and the spectrum was scanned from 270–230 mμ both before and, at a series of times, after the addition of base, the resulting difference spectra (inset to Fig. 2) show the rapid appearance of a peak with its maximum at 245 mμ which slowly declines with time, corresponding closely to that described for acetyl-imidazole.[4] According to the published extinction coefficient of this compound[4] ($\epsilon = 3 \times 10^3$), the observed maximum increase and subsequent decrease at 245 mμ is equivalent to 0.41–0.42 mole acetylimidazole per mole of reactive acetyl in the enzyme. Similar results were obtained in glycine buffer, but in phosphate the magnitude of the change at 245 mμ was reduced.

It is postulated, therefore, that as indicated in Fig. 1, the deacetylation of mono-acetyl-δ-chymotrypsin occurs by a rapid intramolecular transfer of acetyl- from serine hydroxyl to imidazolyl- followed by a slower hydrolysis of acetyl-imidazolyl-. The first order rate constant for the disappearance of the E_{245} compound corresponds closely with that observed for the rate of deacetylation of δ-chymotrypsin as measured by the reappearance of enzyme activity,[6] which in turn corresponds to the rate of base catalyzed hydrolysis of acetyl-imidazole in a model system.[8]

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SYNTHESIS OF POTENTIAL ANTICANCER AGENTS. X. 2-FLUOROADENOSINE[1]

Sir:

Recently the biological activity of three fluoro derivatives of naturally occurring pyrimidines has been reported.[2]

Of these three fluoropyrimidines, 5-fluorouracil and 5-fluoroörotic acid have shown appreciable tumor-inhibitory activity against a variety of rat and mouse tumors[2a] and 5-fluorouracil was selected for clinical trials.[2b] The biological activity of the fluoropyrimidines increased our interest in the preparation of fluoropurines and their ribosides, especially fluoro derivatives of naturally occurring purines. Although Bendich, Giner-Sorolla and Fox were unable to prepare 6-fluoropurine from adenine by the Schiemann reaction,[3] Weisbach successfully prepared 2-fluoropyrimidine from 2-aminopyrimidine by this method.[4] The success of the latter reaction led us to attempt the preparation of 2-fluoroadenosine from 2,6-diaminopurine riboside by diazotization in fluoboric acid.

An aqueous solution of sodium nitrite (360 mg. in 2.4 ml.) was added with stirring to a solution of 2,6-diaminopurine riboside[5] (846 mg.) in 48% fluoboric acid (9.6 ml.) at −10°. The solution was stirred at −10° to 0° for 15 minutes, cooled to −20° and neutralized with 50% sodium hydroxide solution. The water was removed *in vacuo* and the residue chromatographed on a Celite column using water-saturated butanol. The crude 2-fluoroadenosine obtained (149 mg.) was recrystallized from absolute ethanol and dried *in vacuo* over P_2O_5 at 70° for several hours: yield, 75 mg. (8.7%), dec. at 200°; $(\alpha)^{26}D$ −60.3 ± 11.1 (0.127% in ethanol); $\lambda_{max}^{pH\ 1}$ 260.5 mμ (a_M 13,700); $\lambda_{max}^{pH\ 7}$ 260.5 mμ (a_M 14,300); $\lambda_{max}^{pH\ 13}$ 260.5 (a_M 14,800). *Anal.* Calcd. for $C_{10}H_{12}FN_5O_4 \cdot {}^1/_4C_2H_5OH$: C, 42.45; H, 4.60; N, 23.60. Found: C, 42.34; H, 4.93; N, 23.40. A qualitative test for fluorine was positive. The ratio of the R_f values of 2-fluoroadenosine and adenine in butanol–water on a descending paper chromatogram (Watman No. 1) was 0.9.

2-Fluoropurine was prepared in the same manner from 2-aminopurine[6] (850 mg.): yield, 254 mg. (41%) dec. at 216°; $\lambda_{max}^{pH\ 1}$ 264 mμ (a_M 8,300) $\lambda_{max}^{pH\ 7}$ 266.5 mμ (a_M 8,400), $\lambda_{max}^{pH\ 13}$ 272 mμ (a_M 8,800). *Anal.* Calcd. for $C_5H_3FN_4$: C, 43.48; H, 2.20; N, 40.60. Found: C, 43.52; H, 2.01; N, 40.37. A qualitative test for fluorine was positive.

In preliminary tests 2-fluoroadenosine inhibits the growth of Human Epidermoid Carcinoma (HE 2) at 10^{-8} g./ml. Five times this concentration is required to inhibit monkey kidney cells. Azaserine and 6-diazo-5-oxo-L-norleucine inhibit the growth of these tissues at 10^{-7} g./ml.

The preparation of other 2-fluoropurines is now under way in this laboratory.

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(7) Affiliated with Sloan-Kettering Institute.

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THE SYNTHESIS OF 5-FLUOROPYRIMIDINES

Sir:

We wish to report the synthesis of a new class of compounds, some of which were designed to function as nucleic acid antagonists, by substituting fluorine for hydrogen in naturally occurring pyrimidines.

The 5-fluoropyrimidines (III) were obtained from pseudourea and pseudothiourea salts (I) and α-fluoro-β-keto ester enolates (II) by adaptation of the Wheeler synthesis.[1]

Crystalline IIa was prepared by the addition at 0° of 2.4 moles of methyl formate and 1.2 moles of ethyl fluoroacetate (IV) to 1.2 moles of potassium ethoxide in 800 ml. of toluene and letting the mix-

(1) This work was supported by funds from the C. F. Kettering Foundation. Part IX, John A. Montgomery and Carroll Temple, Jr., THIS JOURNAL, in press.

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